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[illegible] AMONG PEDIATRIC Hb S [illegible].

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The number of α globin genes have been documented among 255 active SS patients under the age of 15 years. Ascertainment in the area served by our clinical program is 84%. The common -3.7 Kb α^+-Thalassemia (Th) had a gene frequency of 0.17. The incidence of homozygotes was 2.9%, and heterozygotes 30.6%. These values are consistent with Hardy-Weinberg expectations. There were only two α^+-Th heterozygotes among the 13 children born and deceased in the last 7 years (GF = 0.08). Children with SS;-α/-α had better height/weight parameters (25-50% vs <10% for SS;αα/αα); were less likely to have gallbladder disease (1/8) and more likely to have splenomegaly beyond the first year of life (6/8). Three lines of evidence suggested that α^+-Th interacts with the rapid decline of Hb F in the first three years of life to modify the physical expression of SS: 1. The MCHC of Hb S homozygotes under age 7 appears independent of α gene numbers and thus unlikely to impact on clinical expression; 2. In cord blood, 3 patients with SS;-α/-α had lower Hb Barts (2.4%) than babies with AA hemoglobins and -α/-α (7.2±1.7%); 3. The rate of decline of absolute Hb F values in the first three years of life among children with SS;-α/-α resembles that of patients with SS;αα/αα who maintain a higher Hb F% after the age of 7 yrs (0.121 g/dl/month). The rate of decline of absolute Hb F levels among SS children with αα/αα who will have Hb F under 10% after their 7th birthday is 40% faster (0.172 g/dl/month). These data are consistent with preferential assembly of Hb F over Hb S tetramers during a critical period of postnatal development of SS;-α/-α children. Consequently the inter-cellular distribution of Hb F may be less restricted and help to improve the clinical condition by preserving spleen function. Additional SS newborns with α-Th are being sought to corroborate these observations.

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DEVELOPMENT OF A RAPID IMMUNOASSAY FOR SCREENING ADULT AND NEWBORN BLOOD SAMPLES FOR HEMOGLOBIN S.

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Rapid assays based on solubility have been developed for screening adult blood samples for Hemoglobin S (Hb S). These methods, however, are not applicable to screening newborns, because the low levels of Hb S are easily obscured by high concentrations of Hb F. We have developed a rapid immunoassay for the Hb S screening of both newborn and adult blood samples which combines monoclonal antibody technology in a microtiter well format. The assay utilizes a Hb S-specific monoclonal antibody. Red blood cell lysates are added to individual microtiter wells; hemoglobin present in the lysate adsorbs to the microtiter well surface during a 3 minute room temperature incubation. The solid phase is washed, Hb S-specific monoclonal antibody is added to each microtiter well and incubated for 5 minutes. The solid phase is washed, urease-conjugated anti-mouse Ig is added to each well and incubated for 15 minutes. The solid phase is washed, chromogen is added and allowed to develop color for up to 10 minutes. Microtiter wells containing Hb S develop a distinct blue color. Microtiter wells not containing Hb S remain yellow. No special ancillary equipment is necessary and results can be interpreted by eye. The assay is amenable to automation. The assay described was performed on 800 samples comprised of adult blood, cord blood and filter paper collected newborn heel stick blood. Results correlated 100% to confirmatory electrophoresis. Some of the samples contained other variant hemoglobins such as C, E and D. To date, no other hemoglobin variant has been found that cross reacts with the Hb S-specific monoclonal antibody used in this assay. Whole blood samples, up to 2 months old, containing from 1-30 gm/dl hemoglobin with as little as 1% Hb S can be tested by this assay. In summary, the assay is rapid, easily performed, can be run singly or in batch modes, is amenable to automation, and can utilize both newborn and adult blood samples. The assay can be run in any setting, including doctor's offices, hospital and reference labs, and public health screening centers.

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NOVEL β^S-GLOBIN GENE HAPLOTYPE IN A CAUCASIAN WITH HOMOZYGOUS SICKLE CELL ANEMIA.

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Restriction endonuclease analysis was used to investigate the origin of the β^S-globin genes carried by a Caucasian child with homozygous sickle cell anemia. Despite a high level of hemoglobin F, this patient, of Scotch-Irish-German and Cherokee Indian background from West Virginia and Tennessee, has suffered severe manifestations including painful crises and recurrent strokes. The diagnosis of sickle cell anemia was confirmed by Mst II analysis of patient's DNA which revealed only the 1.4 kb fragment characteristic of the β^S-globin gene and no 1.2 kb fragment characteristic of the β^A-globin gene and most other variant β-globin alleles. β-globin gene haplotypes were determined in the patient and parents for the following polymorphic restriction sites: Hind III ($^G\gamma$), Hind III ($^A\gamma$), Hinc III ($\Psi\beta_1$), Hinc II ($3'\Psi\beta_1$), Hinf I ($5'\beta$), Ava II (β), Hpa I ($3'\beta$), and Bam HI ($3'\beta$). Haplotypes were constructed by examination of familial inheritance patterns of restriction fragment length polymorphisms with respect to the β-globin locus. One β^S-globin gene cluster was demonstrated to contain the Benin haplotype, one of the three common African-type β^S-globin haplotypes. The second β^S-globin gene was present on the most common European haplotype. This gene most likely represents an independent origin of the β^S mutation and is the first reported European-origin sickle cell gene.

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PROTEIN S AND OTHER VITAMIN K DEPENDENT FACTOR CHANGES IN PATIENTS WITH SICKLE CELL ANEMIA.

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The occurrence of large vessel occlusion and previous reports suggest the coagulation system may be activated in patients with sickle cell anemia. Because of speculation that these patients are at risk for thrombosis and the emerging role of natural anticoagulants in preventing inappropriate clotting, we evaluated the levels of protein C, protein S and antithrombin III in 14 asymptomatic HbSS patients compared to 20 normal controls. Free protein S antigen was assayed after polyethylene glycol precipitation. Chromogenic assays were used to determine functional protein C and antithrombin III. Factor VII was assayed in a one stage clotting test and D-D dimer by latex agglutination.

Protein S, protein C and AT III were all significantly lower in the patients than in the controls. The decrease in protein S may reflect

TEST	PATIENTS	CONTROLS	P VALUE*
Protein S (%)	65 ± 31	99 ± 20	<0.005
Protein C (%)	76 ± 19	98 ± 20	<0.005
Antithrombin III(%)	84 ± 13	100 ± 25	<0.025
Factor VII (%)	97 ± 23	103 ± 25	n.s.
D-D dimers (ng/ml)	<200	<200	n.s.

* Student's t, one sided.

true deficiency in free protein S or an increase in C-4b binding protein. The reduction of protein C and S without reduction in factor VII makes changes in vitamin K status or metabolism unlikely. Relative deficiency in AT III, protein C, and protein S may contribute to the pathogenesis of large vessel thrombosis initiated by red cell sickling. Specific therapy to increase these factors may benefit patients with complications of sickle cell anemia.